Discovery, characterization, and kinetic analysis of an alditol oxidase from streptomyces coelicolor

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APPENDIX

DISCOVERY, CHARACTERIZATION AND KINETIC ANALYSIS OF AN ALDITOL OXIDASE FROM *STREPTOMYCES COELICOLOR*

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Running title: Alditol oxidase from *S. coelicolor*

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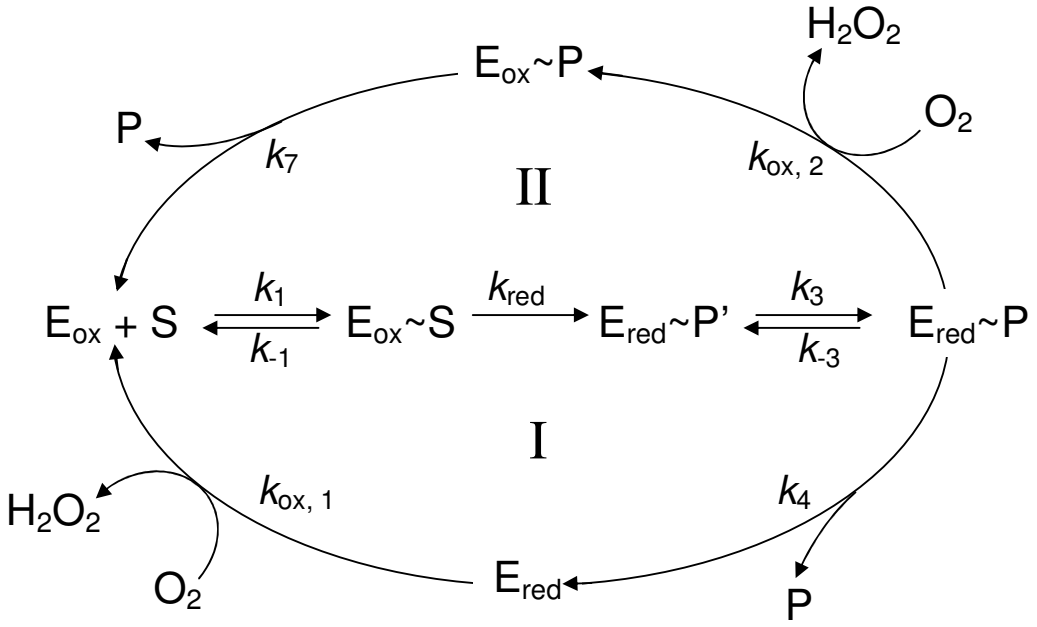
Steady-state kinetics

We used the determinant method for obtaining the steady-state rate equations of the proposed ping-pong and ternary complex mechanisms (1). From these rate equations the steady-state kinetic parameters were calculated. Next to this, we also show the measured and simulated Lineweaver-Burk plots of steady-state kinetics of AldO at varying oxygen and xylitol concentrations. The plots and the calculated steady-state kinetic parameters suggest that AldO follows a ternary complex mechanism.

Scheme 1. Proposed kinetic scheme (I = ping-pong mechanism, II = ternary complex mechanism).



Ping-pong mechanism

To solve the steady-state rate equation for the proposed ping-pong mechanism, the steady-state concentrations of all enzyme species need to be solved. This is accomplished by using the determinant method (1). Because of the relatively fast equilibrium between E_{ox} and E_{ox}~S these species are lumped yielding: $d(E_{ox}+E_{ox}\sim S)/dt = -[E_{ox}\sim S]\cdot k_{red} + [E_{red}]\cdot k_{ox,\ 1}\cdot O_2$ where $[E_{ox}\sim S] = ([E_{ox}] + [E_{ox}\sim S])\cdot S/(K_d+S)$. Substitution of $[E_{ox}\sim S]$ by the latter equation then yields:
$d(E_{ox}+E_{ox}\sim S)/dt = -([E_{ox}] + [E_{ox}\sim S])\cdot S/(K_d+S)\cdot k_{red} + [E_{red}]\cdot k_{ox,\ 1}\cdot O_2$.

$$
\begin{array}{lcccc}
 & E_{ox} + E_{ox}\sim S & E_{red}\sim P' & E_{red}\sim P & E_{red} \\
d(E_{ox} + E_{ox}\sim S)/dt & \left[-k_{red} * \frac{S}{K_d + S} \right. & 0 & 0 & k_{ox,1} * O_2 \\
d(E_{red}\sim P')/dt & k_{red} * \frac{S}{K_d + S} & -k_3 & k_{-3} & 0 \\
d(E_{red}\sim P)/dt & 0 & k_3 & -k_4 - k_{-3} & 0 \\
d(E_{red})/dt & \left. 0 \right] & 0 & k_4 & -k_{ox,1} * O_2
\end{array}
$$

From this matrix the concentration for (E_{ox} + E_{ox}~ S) is obtained by deleting the first row and first column and then calculating the determinant of the remaining smaller matrix. In a similar way the concentrations of the remaining enzyme species can be obtained. These values can then be used to write down the steady-state rate equation in the form of a Michaelis-Menten equation:

$$V = \frac{k_{ox,1} O_2 [E_{red}]}{[E_{ox} + E_{ox}\sim S] + [E_{red}\sim P'] + [E_{red}\sim P] + [E_{red}]}$$

$$V = \frac{k_3 k_4 k_{ox,1} k_{red} O_2 S}{(k_4 + k_{-3}) k_{ox,1} k_{red} O_2 S + k_3 (k_{ox,1} k_{red} O_2 S + k_4 (K_d k_{ox,1} O_2 + k_{red} S + k_{ox,1} O_2 S))}$$

From this rate equation the kinetic parameters K_M and k_{cat} can be derived:

$$K_M = \frac{k_3 k_4 K_d k_{ox,1} O_2}{(k_4 + k_{-3}) k_{ox,1} k_{red} O_2 + k_3 (k_{ox,1} k_{red} O_2 + k_4 (k_{red} + k_{ox,1} O_2))}$$

$$k_{cat} = \frac{k_3 k_4 k_{ox,1} k_{red} O_2}{(k_4 + k_{-3}) k_{ox,1} k_{red} O_2 + k_3 (k_{ox,1} k_{red} O_2 + k_4 (k_{red} + k_{ox,1} O_2))}$$

$$\frac{k_{cat}}{K_M} = \frac{k_{red}}{K_d}$$

Ternary complex mechanism

The steady-state rate equation for the proposed ternary complex mechanism can be derived in a similar way. The result is:

$$V = \frac{k_3 k_7 k_{ox,2} k_{red} O_2 S}{(k_7 k_{red} (k_{-3} + k_{ox,2} O_2) S + k_3 (k_{ox,2} k_{red} O_2 S + k_7 (K_d k_{ox,2} O_2 + k_{red} S + k_{ox,2} O_2 S)))}$$

From this rate equation the following equations for K_M and k_{cat} were derived:

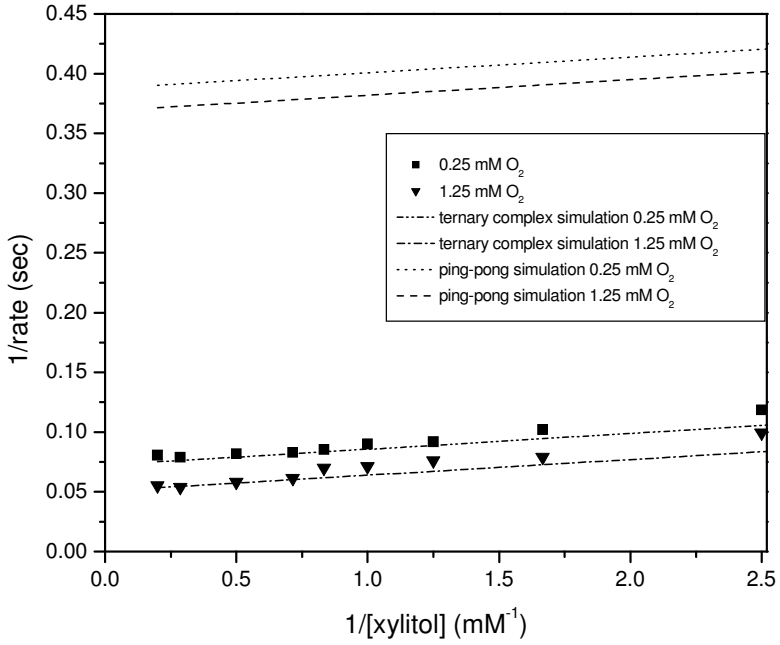
$$K_M = \frac{k_3 k_7 K_d k_{ox,2}O_2}{k_7 k_{red}(k_{-3} + k_{ox,2}O_2) + k_3(k_{red}k_{ox,2}O_2 + k_7(k_{red} + k_{ox,2}O_2))}$$

$$k_{cat} = \frac{k_3 k_7 k_{red} k_{ox,2}O_2}{k_7 k_{red}(k_{-3} + k_{ox,2}O_2) + k_3(k_{red}k_{ox,2}O_2 + k_7(k_{red} + k_{ox,2}O_2))}$$

$$\frac{k_{cat}}{K_M} = \frac{k_{red}}{K_d}$$

Steady-state kinetics of AldO at varying oxygen concentrations

Steady-state kinetic parameters of AldO were determined at 0.25 and 1.25 mM O_2 (k_{cat} is respectively 13 s^{-1} and 20 s^{-1}, K_M is respectively 0.32 mM and 0.49 mM). The set of Lineweaver-Burke plots obtained in this way gives information on the kinetic mechanism that is operative. A set of parallel lines suggests that a ping-pong mechanism is employed by the enzyme, however it is known that in certain cases a ternary complex mechanism yields the same result (2,3). For AldO a set of parallel lines was obtained and correlated to a ternary complex mechanism by simulating the Lineweaver-Burk plot according to the abovementioned equations.



Tested compounds for substrate profiling

D-ribose, L-arginine, diglycerol, L-arabinose, L-alanine, cholesterol, D-xylose, D-alanine, D-lyxose, L-asparagine, Dglucose, L-aspartate, ethyleneglycol, D-mannose, DL-aspartate, diethyleneglycol, D-galactose, L-cysteine, hexaethyleneglycol, L-histidine, PEG, L-proline, D-fructose, L-threonine, 3-buten-1-ol, 3-buten-2-ol, glycerol, sarcosine, cis-2-Butene-1,4-diol, meso-erythritol, DL-homoserine, L-threitol, L-ornithine, ribitol, DL-norvaline, 2-aminoethanol, D-arabitol, 1-amino-2-propanol, xylitol, 2-amino-1-propanol, D-sorbitol, methanol, 4-amino-1-butanol, D-mannitol, ethanol, galactitol, 1-propanol, 2-propanol, 1-butanol, 1,4-diaminobutane, L-rhamnose, 2-butanol, Lfucose, 1-pentanol, 2-pentanol, benzyl alcohol, 1-octanol, 2-phenylethanol, L-gulono-1,4-lactone, isoamylalcohol, α-methylbenzyl alcohol, α-methylbenzyl amine, 1-phenyl-1,2-ethanediol, maltose, 1,2-propanediol, 2-amino-1-phenylethanol, lactose, 1,3-propanediol, 2-amino-2-phenylethanol, sucrose, 1,2-butanediol, 2-amino-2-phenylethanol, 1,3-butanediol benzyl methylether, 1,4-butanediol, cinnamyl alcohol, D-melizitose, meso-2,3-butanediol, 2-methoxy-4-methylphenol, N-acetylglucosamine, butanediol, vanillyl alcohol, butanediol, 1,2-pentanediol, 1,2-hexanediol, cis-1,2-cyclohexanediol.

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